

## Metabolism of Styrene Oxide and 2-Phenylethanol in the Styrene-Degrading *Xanthobacter* Strain 124X

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Received 25 April 1989/Accepted 3 August 1989

Styrene oxide and 2-phenylethanol metabolism in the styrene-degrading *Xanthobacter* sp. strain 124X was shown to proceed via phenylacetaldehyde and phenylacetic acid. In cell extracts 2-phenylethanol was oxidized by a phenazine methosulfate-dependent enzyme, probably a pyrroloquinoline quinone enzyme. *Xanthobacter* sp. strain 124X also contains a novel enzymatic activity designated as styrene oxide isomerase. Styrene oxide isomerase catalyzes the isomerization of styrene oxide to phenylacetaldehyde. The enzyme was partially purified and shown to have a very high substrate specificity. Of the epoxides tested, styrene oxide was the only substrate transformed. The initial step in styrene metabolism in *Xanthobacter* sp. strain 124X is oxygen dependent and probably involves oxidation of the aromatic nucleus.

In the petrochemical industry styrene is produced in large amounts (3.6 million tons in the United States in 1987). It is mainly used as a starting material for synthetic polymers such as polystyrene and styrene-butadiene rubber. It is also used as a solvent in the polymer-processing industry. Consequently styrene is present in many industrial effluents. Especially gaseous styrene emissions often pose a problem because of their malodorous character. However, not all styrene present in the environment is of anthropogenic origin. Styrene can be formed during the decay of organic material due, for example, to the decarboxylation of cinnamic acid (12).

Mammalian styrene metabolism has been studied quite extensively in view of the toxic effects that arise from the metabolic activation of styrene (14). The major pathway of styrene degradation involves styrene oxide as an intermediate. Styrene oxide can subsequently be detoxified in various ways (14).

Contrary to the situation in mammals, no detailed studies have been undertaken to clarify the metabolism of styrene or styrene oxide in microorganisms, although styrene-utilizing bacteria have been isolated (1, 12). As far as we are aware, metabolism of styrene oxide in bacteria has only been reported by Shirai and Hisatsuka (12). They observed that a *Pseudomonas* sp. that was able to grow on styrene had the ability to form 2-phenylethanol from styrene oxide.

In a previous report (15) we described a *Xanthobacter* sp. isolated on styrene. Since we are investigating styrene-degrading bacteria as biocatalysts in biofilters for the removal of styrene from industrial waste gases, we decided that more information concerning the metabolism of styrene is necessary. It would be undesirable if a compound more toxic than styrene (for instance, styrene oxide) would accumulate. The metabolism of styrene oxide and 2-phenylethanol, both potential intermediates of styrene degradation and growth substrates for *Xanthobacter* sp. strain 124X, was therefore investigated.

In this paper, we discuss the enzymes involved in the degradation of styrene oxide and 2-phenylethanol in *Xanthobacter* sp. strain 124X.

### MATERIALS AND METHODS

**Strains and growth conditions.** *Xanthobacter* sp. strain 124X was isolated from an enrichment culture with sewage as the inoculum and with styrene as the carbon source (15). *Pseudomonas putida* LW4 was isolated on D-phenylglycine and has been described previously (16). Strains were maintained on agar slants (7) at room temperature and subcultured every month. Cultures were grown in 5-liter Erlenmeyer flasks in 1 liter of mineral salts medium with 0.1% (wt/vol) carbon source and fitted with rubber stoppers when volatile substrates were used. The flasks were incubated at 30°C on a horizontal shaker oscillating at 1 Hz with an amplitude of 10 cm. The carbon sources 2-phenylethanol, phenylacetic acid, and sodium succinate were added directly, whereas the relatively toxic styrene, styrene oxide, and 1-phenylethanol were initially added in a concentration of 0.01% (wt/vol); after growth was observed, the concentration of the toxic substances was increased step by step until a total of 0.1% (wt/vol) carbon source had been added. The mineral salts medium contained the following (per liter of demineralized water): 1.55 g of  $K_2HPO_4$ , 0.85 g of  $NaH_2PO_4 \cdot 2H_2O$ , 2.0 g of  $(NH_4)_2SO_4$ , 0.1 g of  $MgCl_2 \cdot 6H_2O$ , 10 mg of EDTA, 2 mg of  $ZnSO_4 \cdot 7H_2O$ , 1 mg of  $CaCl_2 \cdot 2H_2O$ , 5 mg of  $FeSO_4 \cdot 7H_2O$ , 0.2 mg of  $Na_2MoO_4 \cdot 2H_2O$ , 0.2 mg of  $CuSO_4 \cdot 5H_2O$ , 0.4 mg of  $CoCl_2 \cdot 6H_2O$ , and 1 mg of  $MnCl_2 \cdot 2H_2O$ . Cells for enzyme purification were grown in a 10-liter Applikon fermentor at a dilution rate of  $0.02\ h^{-1}$  on the mineral salts medium with 4 g of succinic acid per liter and saturated with styrene oxide. The pH was maintained at 7.0. Batch cultures were harvested in the late log phase. Cells were concentrated and washed by centrifugation (7) and either used directly or stored at  $-18^\circ C$ . Cell extracts were made by sonication, and dialysis of extracts was performed with a G-25 column as previously described (7). Culture doubling times were determined by monitoring the increase in  $A_{660}$  with a Vitatron UPS photometer.

**Incubation experiments with whole cells.** Oxygen uptake experiments with washed cells were performed with dilute suspensions of freshly harvested washed cells in a total volume of 3.0 ml of 50 mM potassium phosphate buffer (pH 7.0) at 30°C. After the endogenous oxygen consumption rate was monitored for at least 5 min, 0.1 ml of a 10 mM substrate solution was added, and the oxygen uptake was monitored

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for at least another 5 min. With styrene, styrene oxide, and phenylacetaldehyde saturated solutions (at room temperature in demineralized water) were used instead of the 10 mM substrate solutions. Whole-cell incubation experiments were performed with thawed cells incubated at 30°C in a shaking water bath. Periodically samples were taken and centrifuged for 2 min at  $15,000 \times g$ . The supernatants were analyzed by reversed-phase high-pressure liquid chromatography (HPLC).

**Enzyme assays.** All assays were performed at 30°C with extracts from freshly harvested cells. Activities are expressed in nanomoles of product formed or substrate consumed per minute per milligram of protein. One unit is the amount of enzyme that catalyzes the transformation of 1  $\mu\text{mol}$  of substrate per min.

2-Phenylethanol dehydrogenase (phenazine methosulfate [PMS] dependent) was assayed by determining the increase in the oxygen uptake rate after the addition of 0.1 ml of 10 mM 2-phenylethanol to the reaction mixture (total end volume of 3.0 ml) containing cell extract (0.5 to 1.5 mg of protein)–50 mM Tris hydrochloride (pH 9.0) with 0.1 ml of 10 mM  $\text{NH}_4\text{Cl}$  and 0.1 ml of a 33 mM PMS solution. Inhibition with cyclopropanol was done by adding 50 nmol of cyclopropanol to the reaction mixture.

2-Phenylethanol dehydrogenase [ $\text{NAD(P)}^+$  dependent] activity was determined from  $\text{NAD(P)H}$  formation ( $A_{340}$ ) after 0.2 ml of 10 mM 2-phenylethanol was added to a reaction mixture containing 1.0 ml of 50 mM Tris hydrochloride buffer (pH 9.0), 0.1 ml of 5 mM  $\text{NAD(P)}^+$ , and cell extract (0.5 to 1.5 mg of protein) in a total volume of 1.4 ml.

Phenylacetaldehyde dehydrogenase (PMS dependent) was assayed by determining the increase in the oxygen uptake rate after the addition of 0.1 ml of a saturated solution of phenylacetaldehyde in water to the reaction mixture (total end volume of 3.0 ml) containing cell extract (0.05 to 0.15 mg of protein)–50 mM Tris hydrochloride (pH 9.0) with 0.1 ml of 10 mM  $\text{NH}_4\text{Cl}$  and 0.1 ml of 3.3 mM PMS.

Phenylacetaldehyde dehydrogenase [ $\text{NAD(P)}^+$  dependent] activity was determined in the same way as 2-phenylethanol dehydrogenase activity, except that 0.2 ml of phenylacetaldehyde-saturated water was added to start the reaction in place of 0.2 ml of 10 mM 2-phenylethanol.

Styrene oxide isomerase (SOI) was measured indirectly by measuring  $\text{NADH}$  formation in the presence of cell extract from 2-phenylethanol-grown *P. putida* LW4 (16) containing phenylacetaldehyde dehydrogenase activity. The reaction mixture contained 1 ml of 50 mM potassium phosphate buffer (pH 8.0), 0.1 ml of *P. putida* LW4 dialyzed cell extract (containing 0.3 to 0.5 U of  $\text{NAD}^+$ -dependent phenylacetaldehyde dehydrogenase), 0.2 ml of 10 mM sodium cyanide, 0.1 ml of 5 mM  $\text{NAD}^+$ , enzyme, and water to a total volume of 1.5 ml. The reaction was started by the addition of 0.2 ml of water saturated with styrene oxide. It was always verified that phenylacetaldehyde oxidation was never rate limiting. To determine the pH optimum of SOI, activity was assayed by determining phenylacetaldehyde formation rates (in the absence of  $\text{NAD}^+$ ) by HPLC with a 15-fold purified preparation of SOI prepared by precipitation with ammonium sulfate and gel filtration on an S-300 column. This preparation was also used to determine the substrate specificity of SOI with other epoxides at a concentration of 2 mM.

**Isolation and identification of 2,4-dinitrophenylhydrazones.** Crude extract of styrene oxide-grown *Xanthobacter* sp. strain 124X (5 ml) was incubated with 95 ml of potassium phosphate buffer (50 mM, pH 7.0) containing 3 mM styrene oxide. After the styrene oxide had been completely trans-

formed, the reaction mixture was acidified to pH 1 and centrifuged for 20 min at  $27,000 \times g$  to remove protein. Then 40 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 1 M HCl was added to the supernatant. The resulting precipitate was filtered and dried. The 2,4-dinitrophenylhydrazones of acetophenone and phenylacetaldehyde were prepared in the same way. Mass spectra were recorded on a VG Micromass 3D8 mass spectrometer.

**Partial purification of SOI.** All purification steps were performed at 4°C. The fraction of crude extract precipitating between 15 and 35% ammonium sulfate saturation was collected and dissolved in 5 ml of phosphate buffer (50 mM, pH 7.0). This enzyme solution was applied to a Sephacryl S-300 column (60 by 2.5 cm) and eluted with the same buffer at a flow rate of 30 ml  $\text{h}^{-1}$ . Fractions containing activity were pooled and centrifuged for 1 h at  $150,000 \times g$ . The pellet contained the isomerase activity and was suspended in phosphate buffer (50 mM, pH 7.0).

**Analytical methods.** Protein was determined by the Lowry et al. method with bovine serum albumin as the standard (10). Spectrophotometric assays were performed on a Perkin-Elmer 550A spectrophotometer. Oxygen uptake experiments were carried out with a biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). Headspace analysis of styrene was performed by analyzing 100  $\mu\text{l}$  of the gas phase on a Packard 430 gas chromatograph fitted with a stainless steel column (6 foot by 1/8 in. [ca. 1.08 m by 0.31 cm]) packed with Chromosorb W HP 80-100 mesh coated with 10% Silicone SE-30. The injector temperature was 120°C, the column temperature was 210°C, and the carrier gas was  $\text{N}_2$  at a flow rate of 20 ml  $\text{min}^{-1}$ . Detection was with a flame ionization detector with a detector temperature 230°C, and peak areas were calculated by the internal integrator of the gas chromatograph. Epoxides other than styrene oxide were analyzed by headspace analysis on a Packard 430 gas chromatograph fitted with a Porapak Q column. The oven temperature was 210°C, and the carrier gas was  $\text{N}_2$ . Reversed-phase HPLC analysis was performed at room temperature on a C-18 column (200 by 3 mm; Chrompack, Middelburg, The Netherlands). The eluent was a mixture of Milli Q water and methanol (50:50) at a flow rate of 0.4 ml  $\text{min}^{-1}$ . The  $A_{215}$  was determined by using a Spectroflow 783 absorbance detector (Kratos Analytical, Ramsey, N.J.).

**Chemicals.** Styrene, 1- and 2-phenylethanol, phenylacetaldehyde (containing 20% 2-phenylethanol), and phenylacetic acid were from Janssen Chimica, Beerse, Belgium. Styrene oxide, 1,2-epoxypropane, and ammonium sulfate (biochemical grade) were from E. Merck AG, Darmstadt, Federal Republic of Germany. 1-Phenyl-1,2-epoxypropane and *trans*-2,3-epoxybutane were from Aldrich Chemical Co., Milwaukee, Wis. PMS and styrene glycol were from EGA Chemie, Steinheim, Federal Republic of Germany. Biochemicals were from Boehringer, Federal Republic of Germany, and Sephacryl S-300 and Sephadex G-25 were obtained from Pharmacia, Uppsala, Sweden. Crystallized and lyophilized bovine serum albumin was from Sigma Chemical Co., St. Louis, Mo. All other chemicals were of analytical grade. Cyclopropanol was provided by J. P. van Dijken.

## RESULTS

**Growth experiments.** *Xanthobacter* sp. strain 124X grew with styrene and 2-phenylethanol with doubling times of 19 and 18 h, respectively. The doubling time with styrene oxide was somewhat longer at 23 h, whereas doubling times with

TABLE 1. Oxygen uptake rates of washed-cell suspensions of *Xanthobacter* sp. strain 124X incubated with various carbon substrates

Substrate	Rate of oxygen uptake (nmol of O <sub>2</sub> min <sup>-1</sup> mg of protein <sup>-1</sup> ) <sup>a</sup> by cells grown on:				
	Styrene	2-Phenylethanol	Styrene oxide	Phenylacetic acid	Succinate
Styrene	200	30	15	20	5
2-Phenylethanol	100	100	25	15	<5
Styrene oxide	400	120	250	100	130
Phenylacetaldehyde	350	150	500	130	120
Phenylacetic acid	80	140	150	150	<5
Succinate	ND	40	20	70	125

<sup>a</sup> Rates of oxygen uptake are corrected for endogenous oxygen uptake. ND, Not determined.

1-phenylethanol and succinate were relatively short at 7 and 6 h, respectively. Other potential intermediates of styrene, styrene oxide, and 2-phenylethanol degradation which were utilized as carbon and energy sources by *Xanthobacter* sp. strain 124X were phenylacetaldehyde, phenylacetic acid, and 4-hydroxyphenylacetic acid. Styrene glycol, acetophenone, and 2- and 3-hydroxyphenylacetic acid did not support growth when tested as sole sources of carbon and energy. After growth on styrene and 1-phenylethanol, a yellow coloring of the growth medium was observed.

**Substrate oxidation by washed-cell suspensions.** Styrene degradation by washed cells of styrene-grown *Xanthobacter* sp. strain 124X is oxygen dependent, as was shown by removing the oxygen from the reaction vial by flushing with nitrogen gas before the addition of styrene. Under these conditions no styrene was consumed. After oxygen was added the styrene degradation rate was restored to the same level as in an aerobic control. This requirement of molecular oxygen indicates that the initial step in styrene degradation is an oxidation by an oxygenase. With HPLC analysis, no accumulation of intermediates of styrene degradation was detected when a suspension of styrene-grown cells was incubated with styrene.

2-Phenylethanol-grown cells oxidized phenylacetaldehyde and phenylacetate, the expected intermediates of 2-phenylethanol degradation (Table 1). Interestingly, styrene oxide and phenylacetaldehyde were oxidized by cells grown on all of the substrates tested. The capacity to oxidize phenylacetic acid was limited to cells grown on the aromatic compounds (Table 1).

Incubation of 2-phenylethanol-grown washed cells with 2-phenylethanol resulted in the transient accumulation of phenylacetic acid (Fig. 1A). This 2-phenylethanol-dependent accumulation of phenylacetic acid is a strong indication that 2-phenylethanol is indeed oxidized via phenylacetic acid. With styrene oxide-grown cells a similar transient accumulation of phenylacetic acid as a result of styrene oxide degradation could be detected (Fig. 1B). It would thus seem probable that styrene oxide is also degraded via phenylacetic acid.

**Enzyme activities.** From the above results, 2-phenylethanol metabolism was anticipated to proceed via phenylacetaldehyde and phenylacetic acid. No NAD<sup>+</sup>- or NADP<sup>+</sup>-dependent 2-phenylethanol dehydrogenase activity was detected in extracts from *Xanthobacter* sp. strain 124X; instead, a PMS-dependent 2-phenylethanol dehydrogenase activity was present. This enzymic activity was apparently constitutive and was greatly enhanced by the addition of 0.33

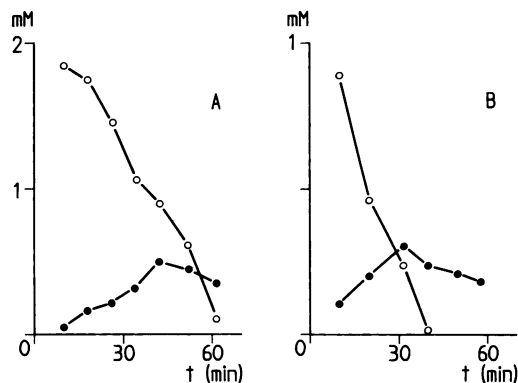


FIG. 1. (A) Transient accumulation of phenylacetic acid (●) from 2-phenylethanol (○) by washed cells of 2-phenylethanol-grown *Xanthobacter* sp. strain 124X (0.8 mg of protein ml<sup>-1</sup>). (B) Transient accumulation of phenylacetic acid (●) from styrene oxide (○) by washed cells of styrene oxide-grown *Xanthobacter* sp. strain 124X (0.6 mg of protein ml<sup>-1</sup>).

mM NH<sub>4</sub>Cl to the reaction mixture (Table 2). Furthermore, increasing the concentration of PMS in the reaction mixture (1.1 mM instead of 0.1 mM) resulted in much higher specific activities. The values for 2-phenylethanol dehydrogenase activity in Table 2 were determined with 1.1 mM PMS in the reaction mixture. 2-Phenylethanol dehydrogenase activity was completely inhibited by the addition of cyclopropanol, which is a suicide substrate for pyrroloquinoline quinone-dependent alcohol dehydrogenases (6).

Both NAD(P)<sup>+</sup>- and PMS-dependent phenylacetaldehyde dehydrogenase activities could be detected in all extracts. Although these enzymic activities appeared to be present in higher amounts in cells grown on the aromatic compounds, they were also present in succinate-grown cells. In contrast to 2-phenylethanol dehydrogenase activity, PMS-dependent phenylacetaldehyde dehydrogenase activity was not enhanced by using higher concentrations of PMS, nor was it inhibited by cyclopropanol. Under the assay conditions used to measure PMS-dependent phenylacetaldehyde dehydrogenase activity, 2-phenylethanol dehydrogenase activity was negligible. This was verified because the phenylacetaldehyde used contained a considerable amount of 2-phenylethanol.

TABLE 2. Specific enzyme activities in crude extracts of *Xanthobacter* sp. strain 124X grown on various substrates

Enzyme	Sp act (nmol min <sup>-1</sup> mg of protein <sup>-1</sup> ) of cells grown on:			
	2-Phenylethanol	Styrene Oxide	Phenylacetic acid	Succinate
2-Phenylethanol dehydrogenase				
PMS dependent	10	<5	5	5
PMS dependent plus NH <sub>4</sub> Cl	250	150	120	100
SOI	55	150	55	18
Phenylacetaldehyde dehydrogenase				
PMS dependent	700	2,200	1,500	420
PMS dependent plus NH <sub>4</sub> Cl	1,000	3,000	1,800	450
NAD <sup>+</sup> dependent	60	150	90	20
NADP <sup>+</sup> dependent	5	15	10	<5

Surprisingly, styrene oxide degradation was detected in crude cell extracts from styrene oxide-grown cells without adding any cofactors. Dialysis of the cell extract did not affect the specific activity of styrene oxide degradation. This apparent cofactor-independent styrene oxide-degrading activity was inactivated by boiling the extract. Enzymatic hydrolysis of styrene oxide to styrene glycol was ruled out, since no styrene glycol formation could be detected and since exogenously added styrene glycol was not degraded under these conditions. The product that accumulated from styrene oxide degradation was identified as phenylacetaldehyde after treatment with 2,4-dinitrophenyl hydrazine and comparing the mass spectrum of the resulting hydrazone with that of the 2,4-dinitrophenyl hydrazone of authentic phenylacetaldehyde. The two mass spectra were identical and differed from the spectrum of the 2,4-dinitrophenyl hydrazone derived from acetophenone. The identity of the enzymatic product of styrene oxide transformation was also confirmed by comparing its retention time and spectrum with those of authentic phenylacetaldehyde by HPLC analysis. The enzymatic activity that catalyzes the isomerization of styrene oxide to phenylacetaldehyde has been designated SOI. Due to the presence of the  $\text{NAD}^+$ -dependent phenylacetaldehyde dehydrogenase activity in crude extracts of *Xanthobacter* sp. strain 124X, SOI could be assayed indirectly by adding  $\text{NAD}^+$  and determining the NADH formation spectrophotometrically. In a separate experiment it was shown that the specific styrene oxide consumption rate was the same as the NADH formation rate. Essential in this coupled enzymic activity assay is the presence of sufficient aldehyde dehydrogenase activity. Routinely, extract from 2-phenylethanol-grown *P. putida* LW4 cells containing 0.3 to 0.5 U of phenylacetaldehyde dehydrogenase was added to the reaction mixture to ensure that the oxidation of the phenylacetaldehyde formed by the SOI activity was not rate limiting. LW4 extract contained no SOI activity. In crude cell extracts no styrene consumption could be detected, either in the presence or absence of oxygen and NADH or NADPH.

**Partial purification and characterization of SOI.** Since SOI represents an epoxide transforming enzyme of a type that, to our knowledge, has not been previously described (see reference 17 for a review), we decided to further characterize the enzyme. SOI was relatively stable, with only a 30% loss of activity after 2 weeks at  $4^\circ\text{C}$  in potassium phosphate buffer (50 mM, pH 7.0). Tris hydrochloride buffers inhibited SOI activity. However, it was not possible to elute SOI activity from a DEAE-Sepharose CL6B column with phosphate buffer and a NaCl gradient. SOI was not inactivated by incubation with similar concentrations of NaCl, thus making it likely that the SOI remained bound to the column material under these conditions. Attempts to assay bound SOI activity by incubating column material with styrene oxide and analyzing for phenylacetaldehyde formation were unsuccessful. Furthermore, SOI activity eluted in the void volume when applied to a column containing the cation exchanger CM-Sephadex. Hydrophobic interaction chromatography with Phenyl-Sepharose was also without success. Only a small amount of SOI activity in a very diluted form could be recovered by using a decreasing salt gradient (1 to 0 M NaCl in phosphate buffer).

Despite the difficulties described above, a partial purification was achieved by ammonium sulfate (15 to 35%) precipitation followed by gel filtration on a Sephacryl S-300 column and ultracentrifugation. This procedure resulted in a total purification factor of 43 and a specific activity of 6 U mg of

TABLE 3. Partial purification of SOI

Prepn	Total activity (%)	Sp act (nmol min <sup>-1</sup> mg of protein <sup>-1</sup> )	Purification factor (fold)
Crude extract	100	142	1
Ammonium sulfate precipitation	73	695	4.9
S-300 gel filtration	72	2,155	15.2
Ultracentrifugation precipitate	72	6,035	42.5

protein<sup>-1</sup> (Table 3). However, the resulting enzyme preparation still contained a considerable number of proteins as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. By using a 15-fold-purified SOI preparation (ammonium sulfate precipitation and gel filtration steps) the pH optimum and substrate specificity were determined. The effect of the pH on SOI activity was determined between pH 6.0 and 8.0 in 50 mM potassium phosphate buffer. In this rather short pH range there was no measurable effect of the pH on SOI activity. Attempts to assay the reverse reaction by incubating the partially purified SOI preparation with various concentrations of phenylacetaldehyde were unsuccessful, indicating that the equilibrium constant favors phenylacetaldehyde formation. SOI seems to have a rather narrow substrate range, since other epoxides tested, including 1,2-epoxypropane, *trans*-2,3-epoxybutane, epichlorohydrin, and 1-phenyl-1,2-epoxypropane, were not transformed.

## DISCUSSION

Growth experiments revealed that the styrene-degrading *Xanthobacter* sp. strain 124X also grew with styrene oxide and 2-phenylethanol. These compounds have both been proposed as initial transformation products in the aerobic degradation of styrene (Fig. 2) (11, 13). Although both styrene oxide and 2-phenylethanol were oxidized by styrene-grown cells (Table 1), we have been unable to obtain

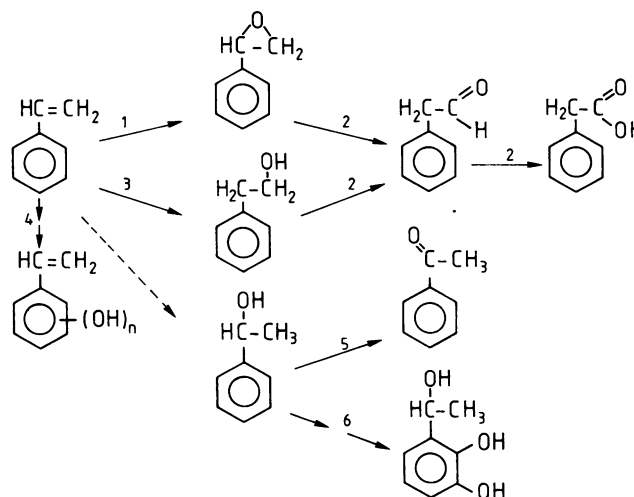


FIG. 2. Bacterial metabolism of styrene and related compounds. Reactions: 1, oxidation of styrene to styrene oxide by a *Pseudomonas* sp. (11); 2, pathways occurring in *Xanthobacter* sp. strain 124X (this study); 3, anaerobic hydration of styrene to 2-phenylethanol (Churchman and Grbic-Galic, Annu. Meet. Am. Soc. Microbiol. 1987); 4, possible oxidation route of styrene in *Xanthobacter* sp. strain 124X; 5, metabolism of 1-phenylethanol in an *Arthrobacter* sp. (2); 6, metabolism of 1-phenylethanol in *Nocardia* sp. strain T5 (2).

evidence for the involvement of these compounds in the metabolism of styrene in *Xanthobacter* sp. strain 124X. The initial oxidation step in styrene metabolism is presently being studied in various other recently isolated styrene-degrading bacteria.

In this paper we have concentrated on the degradation of styrene oxide and 2-phenylethanol by *Xanthobacter* sp. strain 124X. These two compounds are often associated with microbial degradation of styrene (1, 11, 13; J. Churchman and D. Grbic-Galic, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q-32, p. 287).

In *Xanthobacter* sp. strain 124X, 2-phenylethanol is metabolized via a PMS-dependent 2-phenylethanol dehydrogenase activity that was greatly enhanced by the addition of  $\text{NH}_4\text{Cl}$ . This stimulation by  $\text{NH}_4\text{Cl}$  is similar to that observed with the quinoprotein methanol dehydrogenase (5). Cyclopropanol, which has been reported to be a suitable inhibitor of quinoprotein alcohol dehydrogenases in vivo (6), did indeed completely inhibit 2-phenylethanol oxidation by whole cells of *Xanthobacter* sp. strain 124X as well as 2-phenylethanol dehydrogenase activity in cell extracts. Based on the inhibitory effect of cyclopropanol the 2-phenylethanol dehydrogenase from *Xanthobacter* sp. strain 124X is probably a quinoprotein. Recently a quinoprotein alcohol dehydrogenase oxidizing a wide range of primary alcohols was purified from another *Xanthobacter* strain, *X. autotrophicus* GJ10 (8). 2-Phenylethanol was not tested as a substrate for this enzyme, and of the aldehydes tested only formaldehyde was oxidized. No  $\text{NAD}^+$ - or  $\text{NADP}^+$ -dependent 2-phenylethanol dehydrogenase activities were detected in *Xanthobacter* sp. strain 124X, indicating that 2-phenylethanol is metabolized exclusively via the PMS-dependent enzyme. Phenylacetaldehyde oxidation by whole cells of *Xanthobacter* sp. strain 124X and PMS-dependent phenylacetaldehyde dehydrogenase in cell extracts were not inhibited by cyclopropanol, indicating that phenylacetaldehyde and 2-phenylethanol are oxidized by two different enzymes in this strain.

Perhaps more interesting than the 2-phenylethanol dehydrogenase, certainly in view of styrene metabolism, is the novel SOI activity that catalyzes the isomerization of styrene oxide to phenylacetaldehyde. The enzyme was present in all extracts grown on aromatic compounds in a fairly constant ratio with  $\text{NAD}^+$ -phenylacetaldehyde dehydrogenase, indicating that the synthesis of these two enzymes is possibly coordinately regulated. The enzyme was purified 43-fold, resulting in a specific activity of 6 U per mg of protein. Protein purification utilizing anionic exchange proved unsuccessful in the purification of SOI. An explanation for the loss of activity observed in attempts to purify SOI with a DEAE-Sephacrose CL-6B column remains to be found.

The substrate specificity of a 15-fold-purified SOI preparation was very high. We could not detect activity with any of the other epoxides tested. Attempts to assay the reverse reaction were also unsuccessful. However, this is not surprising, considering the difference in the estimated values for the  $\Delta G^\circ$ 's of  $103.5 \text{ kJ mol}^{-1}$  for styrene oxide and  $29.4 \text{ kJ mol}^{-1}$  for phenylacetaldehyde. The  $\Delta G^\circ$ 's were estimated with a group contribution method (9). The resulting  $\Delta G^\circ$  strongly favors the formation of phenylaldehyde.

Based on the analysis of metabolites in urine from rats treated with deuterated styrene, the formation of phenylacetaldehyde from styrene oxide has been proposed previously, although Delbressine et al. assumed that this was the result of a nonenzymic rearrangement of styrene oxide (4).

Perhaps this observation could be ascribed to an SOI activity also being present in rat tissue rather than to chemical rearrangement.

The novel SOI activity we have detected in *Xanthobacter* sp. strain 124X could, if also present in other strains, explain the results of Shirai and Hisatsuka (12), who reported the accumulation of 2-phenylethanol from styrene oxide with a *Pseudomonas* strain. They proposed a styrene oxide reductase activity to explain the formation of 2-phenylethanol from styrene oxide, but 2-phenylethanol formation from styrene oxide in this strain could also be explained by assuming a combination of SOI and 2-phenylethanol dehydrogenase activities being present. The accumulation of phenylacetic acid during growth with styrene by a *P. putida* strain that has been reported by Baggi et al. (1) could possibly, in combination with the assumption that styrene is oxidized to styrene oxide, also be explained by assuming the presence of SOI activity.

An alternative route for the transformation of an epoxide to an aldehyde, which clearly differs from SOI, involves two distinct enzymic activities with a 1,2-diol as intermediate. In *Nocardia* sp. strain A60, 1,2-epoxypropane is hydrolyzed by an epoxide hydratase and subsequently the 1,2-propanediol formed is dehydrated by a 5'-deoxyadenosylcobalamin- and KCl-dependent enzyme, yielding propionaldehyde (3). A combination of two enzymes of this type could also explain the observations of Shirai and Hisatsuka (11) and Baggi et al. (1).

The transient formation of a yellow color, which was observed during growth with styrene and 1-phenylethanol but never during growth with styrene oxide, could be an indication that styrene metabolism in *Xanthobacter* sp. strain 124X has more in common with 1-phenylethanol metabolism than with styrene oxide metabolism. Cripps et al. (2) reported that during growth of *Nocardia* sp. strain T5 with 1-phenylethanol a yellow intermediate appeared. The absorption spectrum of the accumulating compound showed a pH-dependent shift characteristic of a substituted 2-hydroxymuconic acid. After further experiments Cripps et al. proposed a pathway for 1-phenylethanol metabolism in strain T5 involving initial attack of the aromatic ring of 1-phenylethanol by a dioxygenase (Fig. 2). Ring cleavage of the catechol resulted in 2,7-dihydroxy-6-oxoocta-2,4-dienoate formation. Although the spectrum of the yellow color produced by *Xanthobacter* sp. strain 124X was different from that published for *Nocardia* sp. strain T5 (2), the formation of a yellow compound could be an indication that degradation of styrene and 1-phenylethanol in strain 124X also occurs by initial oxidation of the aromatic ring (reactions 4 and 6 in Fig. 2). Further research will focus on the elucidation of the initial step in styrene metabolism in *Xanthobacter* sp. strain 124X and other recently isolated styrene-degrading bacteria.

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